PATENT
CASE 7469/4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Robert M. Lorence
Kirk W. Reichard

Serial No.: 08/260,536

Filed: June 16, 1994

For: METHODS OF TREATING AND DETECTING CANCER USING VIRUSES

Group Art Unit: 1813

Examiner: L. Scheiner

DECLARATION OF DR. ROBERT M. LORENCE

I, Dr. Robert M. Lorence, declare that:

1. I am the same Robert M. Lorence who is named as an inventor on the above-referenced patent application.

2. On page 452 of Reichard et al., J. Surg. Res., 52, 448-53 (1992) ("Reichard et al."), it is stated: "It is unlikely that the effects are due solely to selective infection of rapidly growing cells, since NDV had little effect on rapidly dividing leukemia cells (Lorence and Peeples, unpublished data)." This statement that NDV (Newcastle Disease Virus) had little effect on leukemia cells is based on the in vitro testing of only one leukemic cell line.

3. An experiment was performed as described in Example 2 of the above-referenced application, except that 5

x 10^9 PFU of NDV strain 73-T were used. The NDV was injected intraperitonally into athymic mice to systemically treat subcutaneous IMR-32 human neuroblastoma xenografts.

Complete tumor regression was obtained in eight of ten mice. See attached Figure A. Six mice required a single injection of virus [●] and two mice required two injections of virus [■] to achieve complete tumor regression. Partial tumor regression was noted in the other two mice given NDV [♦, ▲]. No tumor regression was seen in mice given phosphate buffered saline [PBS; □].

The following experiment was performed to demonstrate that the virus was able to reach the tumor and replicate in it after systemic injection. Twenty-four and forty-eight hours after a single intraperitoneal injection of 10^8 PFU of NDV 73-T, mice with IMR-32 neuroblastoma xenografts were sacrificed and the amount of infectious virus in the tumor and various other tissues measured by plaque assay (see Example 1 of the above-referenced application for a description of the plaque assay). The average results of triplicate samples are shown in attached Figure B. After twenty-four hours, infectious virus was only detectable in the tumor and not in any normal organ. Between twenty-four and forty-eight hours, there was an increase in infectious virus within the tumor, but infectious virus was still undetectable in normal tissues.

These results indicate that NDV reaches, selectively replicates in, and then destroys tumor tissue after systemic administration far from the tumor site. Systemic therapy of tumors is conceptually similar to treatment of metastatic cancer. These results, therefore, provide evidence that NDV would also be effective in treating metastatic tumors.

4. I performed calculations to determine the amount of NDV administered to patients in the procedures described in Cassel et al., Cancer, 52, 856-60 (1983), Murray et al., Cancer, 40, 680-86 (1977) and Bohle et al. Cancer, 66, 517-23 (1990). I also compared these amounts to the amounts of NDV used to treat cancer according to the above-referenced application.

Cassel et al. and Murray et al. describe the administration of an oncolysate to melanoma patients by subcutaneous injection to stimulate the immune responses of the patients to their cancers. The oncolysate was prepared by infection of melanoma cells with lytic NDV. To determine the amount of NDV administered to the patients, reference must be made to Cassel et al., Cancer, 40, 672-79 (1977) (hereinafter "Cancer article") which describes the preparation of the oncolysate. The Cancer article states that "the final infectivity titers of the oncolysates ranged from 3.7 to 5.5 logs per 0.05 ml" and that the infectious virus is measured in 50% egg-infectious doses (EID_{50}).

Since Cassel et al. used 2.5 ml oncolysate per injection, the maximum dose given at any one time was 2.5 ml x ($10^{5.5}$ EID_{50}/0.05 ml) = 1.6 x 10^7 EID_{50} per patient, or about 2.7 x 10^5 EID_{50} per kg (assuming a 60 kg patient). For myxoviruses (paramyxoviruses and orthomyxoviruses) such as NDV, 1 PFU = 10 EID_{50}. See Barrett and Inglis, "Growth, Purification and Titration of Influenza Viruses," in Virology, A Practical Approach, page 127 (ed. Mahy 1992) (copy attached). Therefore, Cassel et al. injected 2.7 x 10^4 PFU per kg.

Murray et al. used 2.0 ml oncolysate per injection, so the maximum dose given at any one time was 2.0 ml x ($10^{5.5}$ EID_{50}/0.05 ml) = 1.3 x 10^7 EID_{50} per patient, or

about 2.2 x 10^5 EID_{50} per kg (assuming a 60 kg patient). This corresponds to 2.2 x 10^4 PFU. See Barrett and Inglis.

Bohle et al. describes the intracutaneous administration of an autologous tumor cell vaccine to patients suffering from colorectal carcinoma to stimulate the immune responses of the patients to their cancers. The vaccine was prepared by infecting the tumor cells with a nonlytic strain of NDV. The amount of vaccine given per injection contained 32 hemagglutination units (HAU) of NDV. For myxoviruses such as NDV, 1 HAU = 2 x 10^5 PFU. See Barrett and Inglis. Therefore, 32 HAU corresponds to 6.4 x 10^6 PFU per vaccination, or 1.1 x 10^5 PFU per kg (assuming a 60 kg patient).

Effective amounts for the local treatment of tumors according to the above-referenced application are 4 x 10^8 - 4 x 10^{10} PFU per kg (see page 13, lines 16-19). This is at least 10,000 times more virus (on a kg weight basis) than administered by Cassel et al. and Murray et al. and at least 1,000 times more virus (on a kg weight basis) than administered by Bohle et al. Effective amounts for systemic treatment of cancer according to the above-referenced application are 4 x 10^{10} - 4 x 10^{12} PFU per kg (see page 13, lines 19-22). This is at least 1,000,000 times more virus (on a kg weight basis) than administered by Cassel et al. and Murray et al. and at least 100,000 times more virus (on a kg weight basis) than administered by Bohle et al. In addition, experiments performed by me or on my behalf demonstrate that doses of NDV such as those used by Cassel et al., Murray et al. and Bohle et al. (10^4 - 10^5 PFU per kg) are ineffective for treating cancer when administered systemically.

5. Reichard et al. lists eight authors, including Dr. Kirk W. Reichard and me. Only Dr. Reichard and I would be considered inventors of the subject matter described in Reichard et al. if Reichard et al. were a patent. We conceived of the ideas and designed and interpreted the experiments described in Reichard et al. The other authors listed on Reichard et al. were named as authors because they contributed laboratory space and supplies (Peeples, Walter, Reyes and Greager) or provided technical assistance by performing experiments under the direction and supervision of Dr. Reichard and/or me (Cascino and Fernando).

6. All statements made of my own knowledge are true, and all statements made on information and belief are believed to be true. I am aware that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of this application and any patent issuing thereon.

Robert Lorence

Dr. Robert M. Lorence

Date: June 28, 1995